# Affective startle modulation, verbal and working memory and their relation to CACNA1C genotype.

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## INTRODUCTION

•The CACNA1C gene codes for the pore-forming a1C subunit of the L-type voltage-gated calcium channel, playing an important role in synaptic plasticity, memory formation, learning and behavior.
•The CACNA1C rs1006737 risk 'A' allele is associated with bipolar disorder (BD), major depression (MD) and schizophrenia (SCZ) but clarification of its effects on relevant endophenotypes is required.
• The non-emotional verbal memory (VM) targets hippocampal circuitry and poor performance in this task has been related to genetic risk for BD.
•The non-emotional working memory (WM) task targets prefrontal circuitry and poor performance in this task has been related to genetic risk for SCZ.
• Hypotheses: We hypothesized that healthy homozygotes for the A allele would show reduced performance in VM and relative startle under-reactivity to the affective (pleasant and unpleasant) pictures and reduced performance in the WM task. We also call in question if the gene under study is associated more strongly with mood disorders than with schizophrenia.

## SUBJECTS & METHODS

**Subjects:** The CACNA1C rs1006737 polymorphism was analyzed in 194 unrelated Greek/Caucasian healthy males.

**Quantitative Trait Testing ( phenotyping)**

• Affective startle modulation: The modulation of a startle response during exposure to affective stimuli (pleasant, unpleasant, neutral). It was assessed by magnitude of eye blink startle measured by EMG activity over the area of the orbicularis oculi muscle.
• Neurocognition: Verbal Memory (WMS-III Word Lists task) and Working Memory (N-back task).
• Situational Mood and Feelings: Subjects self-rated the IAPS pictures for emotional valence and arousal using the SAM and their own mood and feelings on arrival to the lab and immediately before the onset of the startle recording using Visual Analogue Scales.

**Genotyping**

Subjects were grouped according to genotype in three groups GG (n=111), GA (n=67) and AA (n=16).

**Statistical methods**

ANOVAs and Kruskal-Wallis tests were used to analyse the phenotypic variables.

## CONCLUSIONS

• Risk A allele homozygotes which has been associated with risk depression and bipolar disorder and with anxious/neurotic traits in healthy subjects, had increased sensitivity to contextual factors such as novelty and anticipation.
• Homozygotes of the A allele had reduced verbal memory performance in the recognition phase, suggesting difficulties with encoding in the hippocampus. The risk individuals did not differ in terms of working memory from the other two genotypes
• The abnormalities of Affective Startle Modulation in the risk individuals are consistent with exaggerated and attenuated activation of their defense and appetitive systems respectively as in anxious/depressed patients, and were predicted by poor Verbal but not Working Memory performance.
• The rating of the emotional pictures as less arousing by the risk A allele subjects is at odds with their psychophysiological responses and may reflect dysregulation of mood appraisal systems.
• The less robust effect of the risk allele on a WM task targeting cognitive processing related to prefrontal cortex and the absence of association between WM and ASM, suggest that this gene has primary effects on amygdala/hippocampal emotional circuitry and thus may be associated more strongly with mood disorders than with schizophrenia.

## RESULTS

**Table 1:** Genotypes did not differ for demographic variables, IQ and state mood on arrival.
[a]Kruskal-Wallis, [b]chi square, [c]one-way ANOVA

| | | GG | GA | AA | P value |
|---|---|---|---|---|---|
| Demographics | Sample size | 111 | 67 | 16 | |
| | Age, years[a] | 22.2±4.0 | 23.3 ±4.5 | 23.6±4.6 | >0.1 |
| | IQ, Raven's raw score[a] | 50.9±9.8 | 50.7± 7.5 | 50.1±8.5 | >0.7 |
| | Smokers/Non-smokers[b] | 44/67 | 23/44 | 8/8 | >0.5 |
| | Smokers: Cigarettes/day | 14.4±7.0 | 14.3±5.8 | 17.8±4.7 | >0.3 |
| Mood on arrival | VAS alertness, cm[c] | 4.92±1.0 | 4.96±1.4 | 4.96±1.0 | >0.9 |
| | VAS anxiety, cm[c] | 2.67±1.5 | 2.21±1.7 | 2.52±1.8 | >0.4 |
| | VAS discontentment, cm[c] | 2.16±1.1 | 1.96±1.3 | 2.12±0.8 | >0.7 |

**Table 2:** We found that the risk A allele homozygotes had poorer performance in the VM recognition phase, consistent with encoding difficulties and more efficient WM (faster reaction times for the same level of accuracy) compared to the other genotypes.

| | | GG | GA | AA | *P* |
|---|---|---|---|---|---|
| Verbal Memory | VM correct responses in recognition | 23.06±1.4 | 22.94±1.4 | 21.87±0.8 | **<0.001** |
| | VM trial 1 recall-List B recall | 0.75±1.8 | 1.32±2.1 | 2.13±1.3 | **<0.006** |
| | VM correct responses in the distractor list | 5.97±1.5 | 5.55±1.7 | 5.19±1.4 | **<0.05** |
| Working Memory | N-Back Accuracy | 13.47±1.7 | 13.46±1.9 | 13.62±1.5 | >0.9 |
| | N-Back Reaction Time | 0.61±0.1 | 0.56±0.1 | 0.58±0.1 | <0.06 |

**Table 3:** Risk A allele homozygotes (AA) became more anxious and discontent immediately after instructions and prior to startle testing, suggesting higher contextual sensitivity, compared to the other genotype groups.

| | | GG | GA | AA | *P* |
|---|---|---|---|---|---|
| VAS mood pre-test | Anxiety | 2.69±1.4 | 2.56±1.7 | 4.87±1.4 | **<0.001** |
| | Discontentment | 2.32±1.3 | 2.01±1.2 | 2.91±1.2 | **<0.02** |
| | Alertness | 4.30±1.0 | 4.50±1.1 | 4.52±0.9 | >0.1 |
| VAS mood post-test | Anxiety | 2.57±1.5 | 2.30±1.4 | 2.74±1.5 | >0.5 |
| | Discontentment | 2.25±1.1 | 2.13±1.2 | 2.76±1.2 | >0.4 |
| | Alertness | 4.20±1.2 | 4.27±1.2 | 3.91±1.2 | >0.3 |

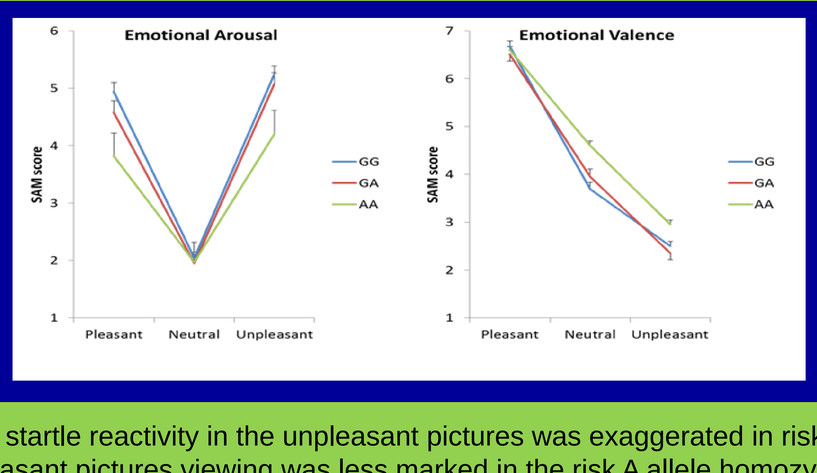
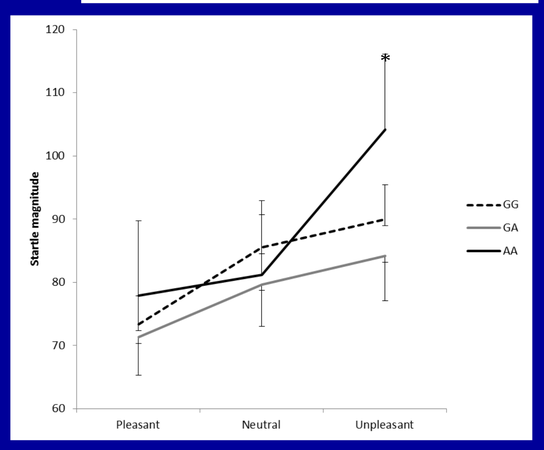



**Figure 1:**Risk A allele homozygotes rated the IAPS affective pictures as less emotionally arousing.

**Figure 2**:The normal pattern of high startle reactivity in the unpleasant pictures was exaggerated in risk A allele homozygotes (AA). The normal startle attenuation during pleasant pictures viewing was less marked in the risk A allele homozygotes**.**

Startle response in all viewing conditions




Difference from Neutral